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13. ABSTRACT ***(Maximum 200 Words)***

Our hypothesis is that functional redundancy in Base Excision repair (BER) may permit polymorphism to accumulate in these parallel pathways. Deficiencies in BER may lead to elevated spontaneous mutation rates and an earlier onset of cancer. We have analysed two BER enzymes: TDG and MED1(MBD4), both DNA N-glycosylases that remove the T residue in a T/G mismatch and the U residue in a U/G mismatch. Our analysis revealed that neither MED1 nor TDG polymorphisms appear to be related to breast cancer of the general population. Curiously, compounded heterozygous exon 5 and exon 10 polymorphisms of the TDG gene, in our analysis of 590 patients is under-represented. It may imply that the TDG gene has a second role in humans besides DNA repair, and that the presence of both alleles in a person may lead to embryonic lethality. The latter can be a checkpoint for eliminating defective gene coding for defective enzymes that may be damaging later in life. We observed that the exon 10 polymorphism of TDG is unusually high in frequency in the high risk ovarian population. This potentially important finding needs further examination with a larger population in the future.

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Base Excision Repair Gene Mutations and Polymorphisms as a Potential Modifier of Breast Cancer Risk

INTRODUCTION:

The original focus of this research is to evaluate the possibility that DNA polymorphisms that changes the amino acid sequences of the enzymes of base excision repair (BER) may be related to cancer. BER is one of three major ubiquitous DNA repair systems protecting all cells. Lesions repaired by BER include the oxidation of DNA by oxygen, and the result of the natural instability of the bases of DNA, leading to the deamination of cytosine and 5-methylcytosine, and the opening of the ring structures in DNA bases. We focus on BER systems for two important reasons. First, unlike carcinogens, the DNA lesions repaired by BER occur spontaneously in each cell thousands of times per day such that the pressure towards mutations is always present. If BER were compromised, a cascade of gene damages may lead to cancer. Secondly, the cell uses two BER enzymes against each spontaneous lesion to provide extra safety. Our hypothesis is that this functional redundancy in BER permits the opportunity for mutations and polymorphism to accumulate in these parallel pathways. Sporadic cancers may be associated with germline defects in different genes of BER. Because there are many BER enzymes, the association of each one with breast cancer may appear to be sporadic instead of familial. Having two undesirable BER polymorphisms/mutations in the same person would be much worst than having just one.

We proposed to examine four BER genes, MED 1 and TDG that are the first enzymes in the pathways for detecting and repairing cytosine deamination reactions, and OGG1 and hMYH that are for repairing the damage and mutations produced by the oxidative damage lesion 8-oxoguanine.

We proposed to include two hundreds disease-free women as our reference control group. The three test groups will consist of (i) two hundred non-BRCA1/2 women (or maximum number available) with sporadic breast cancer, (ii) two hundred (or maximum number available) BRCA1/2 women without cancer, and (iii) two hundred (or maximum number available) BRCA1/2 women with breast cancer. We planned to screen their germline DNA for the presence of BER gene defects in the coding regions, and to test for the correlation of these BER gene defects with breast cancer in the presence or absence of BRCA1/2 mutations.

Over the course of one year support of the Concept award, and the second year of no cost extension, we made some changes in our plans to accommodate the lessons we learnt, and to strengthen the findings that we feel are most significant. Our studies focused on the MED1 gene and the TDG gene, with the patient population reaching the 800 mark that we hoped to achieve. To finding meaning in the data, we had to genotype for genes outside of the TDG gene loci to distinguish different haplotypes. That broadening of this task was by itself more than the Concept award funding could support. Thus although the OGG1 and hMYH 1 tasks were started, they remain unfinished until we can secure

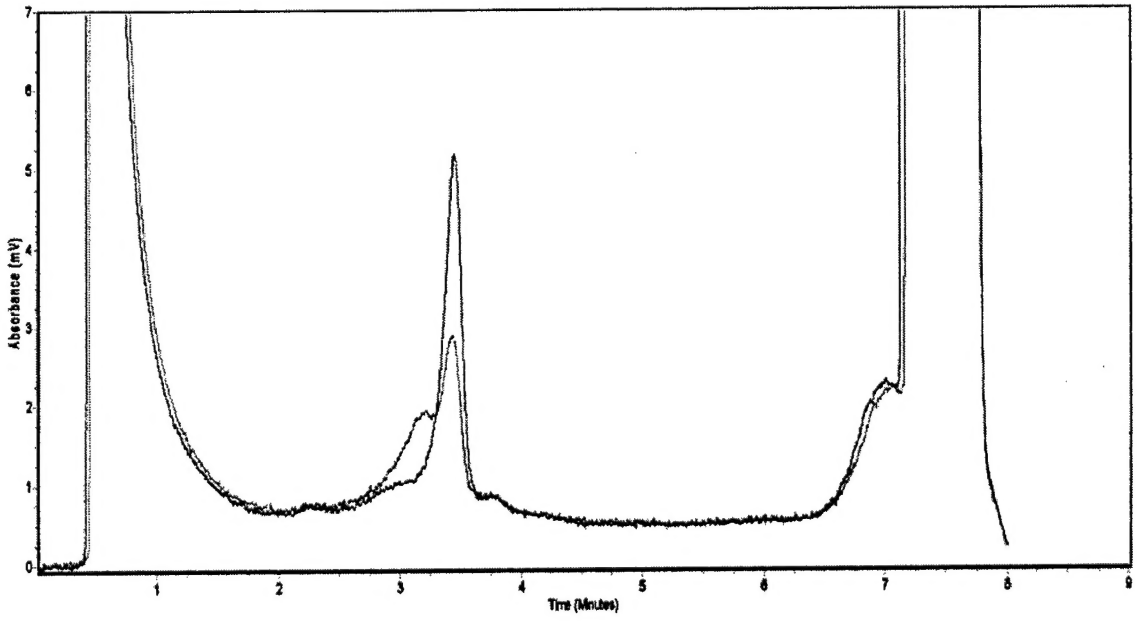
further funding support. Our concept of BER polymorphisms has been enhanced to include the possibility that damage to adult life by BER defects may be eliminated in nature by screening for BER defects during early development by reusing BER enzymes in a secondary essential developmental role. Because this is a report of the no cost extension year, only new progress is reported.

BODY:

In year 1, we developed our methods of polymorphism screening and screened 268 patients for polymorphisms in MED1 and TDG genes. Here I report describes the progress with respect to the area of investigation described in my extension proposal.

Task 5: Months 13-16 Screening 500 research participants for polymorphisms in TDG
We will screen for the exon 5 Ser and exon 10 Met polymorphisms in the DNA of 500 more patients.

We acquired a DHPLC WAVE system (Transgenomic Inc.) and optimized it for the detection of the known polymorphisms in the TDG gene. The WAVE system is an ion pair HPLC reversed phase system running a propriety column at 50-80 C. It is sensitive to DNA secondary structures and can be used for the optimization of PCR conditions and distinguishing homoduplexes from heteroduplexes. As shown in the center of the figure below, the homoduplex of the TDG exon 5 polymorphism shows a single peak while the heteroduplex trace shows two peaks.



364 new patients were screened for TDG polymorphisms blind, separated in four plates. The results on the TDG exon 5 polymorphism is shown in the following figure:

	Mt/wt (heterozygous)	%	Mt/mt (homozygous)	% of mutant allele
Plate 1 (70 samples)	1	1.5%	N.D.	0.75%
Plate 2 (74 samples)	7	9.5%	N.D.	4.7%
Plate 3 (80 samples)	3	3.9%	N.D.	1.9%
Plate 4 (100 males)	12	12%	N.D.	6%
Total (324 samples)	23	7.1%	N.D.	3.5%

Similarly, we screened these 324 patients for their polymorphisms in the TDG exon 10. The results are as follows:

	Mt/wt (heterozygous)	%	Mt/mt (homozygous)	% of mutant allele
Plate 1 (70 samples)	22	31.4%	1/43 (rest .D.)	17.1%
Plate 2 (74 samples)	7	9.5%	1/56 (rest .D.)	6.1%
Plate 3 (80 samples)	13	16.3%	1/78	9.4%
Plate 4 (100 males)	13(/98)	13%	N.D.	6.5%
Total (324 samples)	55 (/322)	17.1%	3 (177)	9.4%

The results are decoded and summarized as follows:

	% exon 5 mt/wt patients	% exon 10 mt/wt patients
Plate 1: High risk ovarian	15 %	31.4 %
Plate 2: Female breast cancer	9.5 %	9.5 %
Plate 3: Control females	3.9 %	16.3 %
Plate 4: Control males	12 %	13.6 %

The analysis of the overall results of all the patients is as follows:

To gain statistical power, we have totaled research participants to 592. Although another 200 patients are currently being screened to reach a total of about 800 patients, their results are not yet processed for description in this report. The table shows the distribution of the genotypes and the allelic frequencies in this population. One research participant was found to harbor one mutant allele at each polymorphic site. Our hypothesis is that different haplotypes of the TDG region can be found and it is only the combination of some of them that is lethal. This year, we learnt more about how to calculate the allele frequencies more precisely:

For the 268 patients of the study in year 1:

Genotype	Observed	Expected
5/5 10/10	199	202.6
5/5 10/10	46	43.5
5/5 10/10	20	19.1
5/5 10/10	2	2.3
5/5 10/10	1	0.4
5/5 10/10	0	2.1

Smaller lettering = polymorphism

Allelic frequency of exon 10 polymorphism = 0.093
Allelic frequency of exon 5 polymorphism = 0.041

For the combined 592 patients in both years:

Genotype	Observed	Expected	$(obs-exp)^2/exp$
5/5 10/10	444	449.7	0.072
5/5 10/10	100	96.7	0.113
5/5 10/10	41	39.2	0.083
5/5 10/10	5	5.2	0.008
5/5 10/10	1	0.9	0.011
5/5 10/10	1	4.2	2.44
			Chi ^{2} = 2.73

Allelic frequency of exon 10 polymorphism = 0.094
Allelic frequency of exon 5 polymorphism = 0.038

Conclusion:

We realized during data analysis in this study that even in a population of 800 patients, one does not have enough statistical power to prove linkage of a polymorphism with a disease if the polymorphism is also found in the normal subjects at an appreciable frequency. For example, a polymorphism of 5% in the disease population but 1 % in the normal population would be inconclusive, whereas a 25% polymorphism in the disease and 1% in the normal would suggest that it affects a subset of the disease population. When considering the linkage of two polymorphisms as initially conceived in this proposal, only two polymorphisms of the 20 % range can be linked conclusively with this size population, and even there, the appearance of the linkage in the normal population must be very low to allow statistical significance. This reality essentially eliminated the usefulness of finding rare polymorphisms in our population, unless they are tightly linked within a family.

The data reported in this study do not support a role for MED1 and TDG polymorphisms, by themselves, as determinants of a significant fraction of ovarian cancer (for MED1 and TDG) or breast cancer (for TDG). The combination of polymorphisms in MED1 and TDG does not appear to be significant either. Whether these polymorphisms may serve that role in combination with the polymorphisms of other base-excision repair genes will be revealed in future studies.

The two frequent polymorphisms observed in the TDG gene are observed within the same person with a frequency lower than expected. A more vigorous statistical analysis is necessary. If the observation holds true for a larger population, it would suggest that the coexistence of both alleles may, under certain conditions, be embryonic lethal, which is now known for homozygous TDG knockouts in mice. In our case, the polymorphisms in TDG we followed may be functional in themselves, or serve only as a marker for another more serious mutation in another gene in the faplotype region. Haplotype mapping of the chromosomal region around the TDG allele will be used to investigate which gene may be responsible for this effect.

We have expanded considerable effort in this direction to genotype the genes around TDG in an attempt to uncover the meaning of the separation of the exon 5 and exon 10 polymorphisms in our test population. While the findings are preliminary, we observed that there are multiple haplotypes for the TDG region such that the TDG exon 5 and exon 10 polymorphisms can either be of importance in themselves, or merely serve as markers for other more important polymorphisms in that region of the chromosome. These distinct haplotypes, if confirmed, may provide interesting insight into human evolution and migration, as well as provide an explanation for the apparent segregation of the TDG polymorphisms.

Task 6: Months 13-25 <u>Screening research participants for polymorphisms in OGG1</u>
The second pair of BER enzymes to examine are OGG1 and hMYH. OGG1 is a DNA N-glycosylase that repairs the oxidation lesion of guanine by removing the 8-oxoguanine residue from an 8-oxo-G/A mismatch. hMYH is the human homolog of the E. coli MutY gene. It is a DNA N-glycosylase that removes the A residue that is misincorporated

across an 8-oxo G residue present in the oxidized template strand. Some OGG1 polymorphisms are known to affect enzyme function.

We have collected the information on all known polymorphisms of the OGG1 gene that causes changes in the amino acid sequence.

Codon	Position	DNA Alteration	AA Alteration
46		G to A	Arg to Gln
85	561	G to T	Ala to Ser
98		G to A	Lys to Lys
131	730	G to A	Arg to Gln
154		G to A	Arg to His
232	1032	T to A	Ser to Thr
308	1231	G to A	Gly to Glu
326	1285	C to G	Ser to Cys

We performed genotyping of three of these polymorphisms in about 20 patients and found them in both controls and disease subjects, thereby suggesting early on that even the analysis of a population of 800 subjects will be inconclusive. Yet one does not know, unless one accumulates enough polymorphism observations in the normal subjects, that the initial observations in the normal subjects is a chance clustering of rare events. Some of these alleles are difficult to PCR to good quality, and will require more work to have reliable data. We have exhausted our capacity under this proposal extension to perform the genotyping of this many polymorphisms in our panel of patients. Therefore this task will await our obtaining other funding before it can be completed.

Task 7: Months 13-25 Screening research participants for polymorphisms in hMYH
We have exhausted our capacity under this proposal extension to perform the genotyping of these polymorphisms in our panel of patients. Therefore this task will await our obtaining other funding before it can be completed.

PERSONNEL SUPPORTED BY THIS GRANT:

Anthony Yeung, Principal Investigator: 5% FTE.

Sara Griffith, B.S. Technician, 50% FTE.

KEY RESEARCH ACCOMPLISHMENTS:

Year 1:

1. The MED1 gene has only one known high frequency DNA polymorphism, and apparently no rare polymorphisms in our population.
2. We confirmed that the TDG gene has only two known high frequency DNA polymorphisms and apparently no rare polymorphisms in our population.

3. The two frequent polymorphisms, as a compounded heterozygous, appear to be under-represented within the same person, suggesting that coexistence of both alleles may be embryonic lethal. The condition may further suggest that the TDG gene has another role in early development independent of its known role in DNA repair.

Year 2:

1. The two polymorphisms of the TDG gene continue to show some preference not to coexist in the same person.
2. High-risk ovarian patient population seems to have higher frequencies of TDG exon 10 polymorphisms.
3. By examining the haplotype of SNPs outside of the TDG gene, it appears that humans are divided into just a few major groups with respect to this gene region.

REPORTABLE OUTCOMES:

None yet

CONCLUSIONS:

The original hypothesis that having parallel pathways may lead to accumulation of deleterious mutations in base excision repair genes has to be modified to suggest that in some cases, some polymorphisms may be eliminated early in development to prevent deleterious effects later in life. In the case of the MED1 gene and the TDG gene, theirbiotechcelery functional overlap has not led to detectable functional polymorphisms in the MED1 gene. It is not clear whether TDG polymorphisms, even as our data suggest that they have important new roles in the cell, arose because of functional overlap with the MED1 gene product. This possibility may be testable with future gene knockout experiments. Available data in this study does not support a role for MED1 and TDG polymorphisms, by themselves, as determinants of a significant fraction of breast cancer. Whether they may serve that role in combination with the polymorphisms of other base-excision repair genes will be revealed in future studies.

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4. Meeting Abstract: Era of Hope meeting, Sept 25-29, 2002 at Orlando, Florida. Poster Title: Base Excision Repair Gene Mutations And Polymorphisms As A Potential Modifier Of Breast Cancer Risk

BIBLIOGRAPHY OF PUBLICATIONS AND MEETING ABSTRACTS:

None

APPENDICES:

None